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The development cycle of *Posthodiplostomum brevicaudatum* (v. Nordmann, 1832) Kozicka, 1958

Cykl rozwojowy *Posthodiplostomum brevicaudatum* (v. Nordmann, 1832) Kozicka, 1958

In 1950 and 1951 Kozicka (1958) found in the fry and fish of various species (*Leuciscus rutilus* L., *Perca fluviatilis* L.) and above all in *Scardinius erythrophthalmus* L. of Lake Drużno the metacercaria of *Posthodiplostomum brevicaudatum* (Nordmann, 1832) Kozicka, 1958 living in a cyst in the eyes and the brain. This larva is common not only at Lake Drużno and was recorded by the same author also at other lakes of the Mazury District (Łabędzie, Gołpapiwo and Mamry Północne) although in distinctly smaller numbers. Outside Poland it was found in Germany and the Soviet Union and described by Nordmann (1832) as *Holostomum brevicaudatum*, by Diesing (1850) as *Diplostomum brevicaudatum*, by Dogiel and Bychowskij (1934) and Zachwatkin (1940) as *Neascus brevicaudatus*.

A considerable number of piscivorous birds were examined at Lakes Drużno, Gołdapiwo and Mamry Północne, where the metacercaria was found in fish, at the same time between 1950 and 1957.

Although a comparatively high number of metacercariae occurred in fry and fish no equivalent of mature forms was found in such piscivorous birds as *Lariformes*, *Pelecaniformes*, *Podicipidiformes*, *Ardea cinerea* and *Alcedo atthis*. Only a form which could be compared with the metacercariae found in fish was discovered in *Botaurus stellaris* and *Ixobrychus minutus* at Lake Drużno and Lake Mamry Północne. I denoted that form as *Posthodiplostomum impraeputiatum* Dubois, 1934, first found in France also in *Botaurus stellaris*. Through its being discovered in *Ixobrychus minutus* the list of hosts of this fluke was expanded.

Table I

Piscivorous birds examined

	Drużno 1950—51	Gołdapiwo 1954—55	Mamry Półn. 1956	Total	Number of	
					birds invaded	*P.impraeputiatum*
Larus ridibundus	23	12	26	61	—	—
Sterna hirundo	42	8	6	56	—	—
Chlidonias nigra	18	—	10	28	—	—
Ardea cinerea	6	14	—	20	—	—
Phalacrocorax carbo	1	1	5	7	—	—
Podiceps cristatus	16	52	37	105	—	—
Podiceps griseigena	1	5	1	7	—	—
Alcedo atthis	3	—	—	3	—	—
Botaurus stellaris	1	—	1	2	1	37
Ixobrychus minutus	2	—	—	2	2	8

All doubts as to whether the metacercaria belonged to *P. impraeputiatum* could have been removed by experiment but it had proved impossible to feed the final host with the metacercariae as it was extremely difficult to procure live *B. stellaris* and *I. minutus*. The only practicable way of establishing the fragment of the life cycle between the fish and the bird was morphologically to identify of the metacercaria with the mature form discovered in nature. But to relate other fragments of the life cycle by experiment remained an open question.

I found the cercaria supposed to belong to *Posthodiplostomum* in the biocoenoses in which I discovered the metacercaria and the mature form. I found it at Lake Drużno in 1953 and at Lake Mamry Północne in 1956, *Planorbis planorbis* L. being the host at both. Attempts at invading young fish, up to one year (0+) and up to two years (1+) of age gave positive results. In them, I obtained metacercaria stages repeatedly recorded before under the name of *Neascus brevicaudatus*. It was easy to identify them with the metacercaria found in fish at Lakes Drużno and Mamry Północne.

As I had both experimental and natural material in large quantities at my disposal I was able to determine the characteristic morphological properties of the metacercaria during its development.

The established development cycle was thus partly based on experiment (cercaria — metacercaria) and partly on a morphological comparison of the stages occurring in fish and birds (metacercaria — mature form). The final identification was decided by a very essential feature, i. e. the structure of the copulative apparatus, found in the grown-up metacercaria and the mature form. Flukes with a similar structure of this part of sexual apparatus were found only in *B. stellaris* and *I. minutus*. Accordingly, although there is no experimentally established link at the last stage of the development cycle the passage from *Planorbis planorbis* through fry or mature fish to *B. stellaris* and *I. minutus* may be regarded as determined beyond any doubt. And as it was established that *Posthodiplostomum brevicaudatum* is the metacercaria of the fluke *Posthodiplostomum impraeputiatum* Dubois, 1934 the name of *Posthodiplostomum impraeputiatum* Dubois, 1936 should be changed because of priority to *Posthodiplostomum brevicaudatum* (v. Nordmann, 1832) Kozicka, 1958.

The cercaria

The cercaria *P. brevicaudatum* ranks with those furcocercariae which are found comparatively seldom in the biocoenosis. Among 10,255 specimens of various snail species examined between 1950 and 1953 at Lake Drużno I discovered it only six times in 1953. It was my special objective to find that cercaria in natural condition as I undertook to determine the development cycle. In my quest for the cercaria I collected snails from the thicket, shrubs and reeds on the lake where there occurred *Botaurus stellaris, Ixobrychus minutus* and *Alcedo atthis,* then supposed to carry the mature parasite. Among the snails I collected at such sites I found in *Planorbis planorbis* a cercaria very closely resembling *Posthodiplostomum minimum* (McCal) (= *Cercaria multicellulata* Müller), *C. hamata* Müller, 1923 and *C. rhabdocaeca* Faust, 1919. It proved to be cercaria *P. brevicaudatum.*

Although the dimensions show that it is a long cercaria, it may be classified as a small form owing to its body being narrow: 0.15 × × 0.046 — 0.30 × 0.015 + 0.18 + 0.21 mm. The dimensions are given here in the following order: length of body by width + length of tail trunk + length of furca.

Noteworthy is a great contractility of the cercaria through which its length and width may be made twice as large or small. The cercaria is usually narrow and long as a rod and only its terminal section has a bell-like expension. When contracted, it does not change its rod-like shape but is shorter and wider. The dimensions of the body are then 0.18 — 0.25 × 0.15 — 0.03 mm.

The characteristic feature of the cercaria is that its cuticle wrinkles behind the pharynx, the wrinkles disappearing only with a stronger contraction of the body. The frontal organ (0.04 × 0.025 — 0.06 × × 0.02 mm) has delicate contours. No muscles are visible and only expanded terminal sections of the penetrative glands and the armature may be traced. The armature of the head consists of eight to ten rows of fine spines, the dimensions of which diminish as they recede from the top of the head. The spines disappear in the hind half of the frontal organ (Fig. 2). The head never stops moving. Its top extrudes or hides during a contraction. A hollowing appears then in which the spines are crowded (Fig.) only to be thrust vehemently outside in a moment. These movements must be of importance when the cercaria penetrates the body of a fish and when it wanders about the body. They help the mechanical action of the spines and the secretion from the penetrative glands.

A praepharynx of a narrow lumen passes through the frontal organ. At a distance behind the frontal organ it is surrounded by a group of muscles forming the anlage of the future pharynx. The further section of the alimentary tract has very weak contours and disappears at the height of the first cells of the penetrative glands. It corresponds to the oesophagus in position. No intestinal branches appear yet; they form in the metacercaria stage only.

The penetrative glands occur as three pairs of cells. They lie in the hind part of the body and occupy the middle section of it. The cells are large, the nuclei distinct, the plasm finely granulated, all of them showing clearly against the parenchymatous cells. Although the glandular cells occupy the hind section of the body they do not reach its end. Each cell passes into an efferent duct stretching towards the frontal organ. After they have entered inside the organ the ducts expand considerably and terminate at its top among the spines.

Between the first and second pairs of glandular cells there appears a group of cells which may be regarded as an anlage of the ventral sucker. It corresponds in position to that organ in other fur-

cocercariae, but although it always occur it has no distinct morphological features of the sucker. It is covered by the first or second pair of glandular cells and consequently it is not distinct and may easily escape attention.

Between the penetrative glands and the excretory vesicle there is an anlage of the future reproductive organs in a form of a compact group of cells. No special arrangement can be distinguished among them and only their persisting in the metacercaria all through the period of morphogenesis warrants such interpretation.

Sideways half through the length of the body in front of the penetrative glands are two small eye spots consisting of a few grains of dark pigment. The grains are situated on the circumference of the vesicle which has the shape of a lens.

The formula of the excretory system is $2\,[(2 + 2 + 2) + (2 + + 2 + 2)] = 24$. The vibratory flames lie symmetrically on both sides of the body in pairs. The first pair lies behind the frontal organ, another between the eye spots and the first pair of glandular cells. Three more pairs lie in the hind part of the body, the third pair lying at the height of the anlage of the ventral sucker. The last pair is in the trunk of the tail. I saw only one „flame" in each of the two pairs but one. The excretory vesicle is small and consists of two parts, front and hind. The division disappears when the vesicle is filled up. I observed no „Cort's islet".

The tail differs from the body only slightly in size. Its width is about the same as that of the body, except for the bell-shaped expanded section. It is only somewhat shorter than the body. The strations reach the length of the trunk or a little farther. Almost the whole of the trunk is filled with five pairs of large caudal bodies. They lie probably on the surface, for when the cover glass is strongly pressed they disappear and numerous cells stretching down the middle of the trunk of the tail from the base of the tail to where it forks into furcae. These are muscular cells, longitudinal or circular species, differing from it by smaller dimensions and by the

In the middle of the trunk runs the excretory canal, an extension of the excretory vesicle. It bifurcates and enteres each of the furcae where it terminates with an opening half through its length.

The cercaria develops in long sporocysts and in some snails I found large numbers of it.

In addition to the cercaria described I discovered a very similar species, differing from it by smaller dimensions and by the appearance in *Physa fontinalis* L. The number of cercariae of the species occurring is distinctly smaller than that of *P. brevicaudatum*. The experiments carried out show that the cercaria from *Planorbis planorbis* is a larva of *P. brevicaudatum*, while that developing in *Physa fontinalis* is most probably a cercaria of *Neascus scardinii* (Schulmann) found in the fish brain.

The invasion of fish by cercariae

The cercariae leave the snail in the morning. They show a positive phototropism and tend towards the upper layers of the water but do not accumulate just under its mirror. Their behaviour harmonizes with the way of living of young fry (15 to 55 mm long) which also gathers in great numbers in the upper layers of the water.

When the fishes are put into the vessel the cercariae soon gather around them and atack them. The cercariae penetrate into the young fishes very rapidly. The penetration into older fishes the skin of which is covered by scales takes much longer as the scales resist it. I found after 20 minutes in the muscles of the fishes several to a few scores cercariae differing from the forms swimming in water only in that they have no tail. They occurred in various parts of the body, mainly in the trunk and wandered quickly towards the head.

They do not choose where to penetrate into the fish. They penetrate into the muscles of the trunk on the dorsal and ventral sides, the pectoral, trunk and caudal fins, the head, the eyes etc. They are to be found in the skin, the subcutaneous connective tissue, the vitrous body of the eye or in the nervous tissue of the brain within a few hours of the contact of the fish with the cercariae. They may also be seen sticking immobile in the skin. Although they have no predisposed region of the body they tend in the direction of the eye as directly as possible after they penetrate the body. For all obstacles they are very persistent and pierce through the muscular, connective, nervous tissues and through the blood vessels. Yet they do not use the blood system for passage. With the help of battering movements of their armed head and probably using the secretion of penetrative glands they move through the tissue to the eye and settle in the chorioid coat or the retina. They may also wander from the eye

to the brain. Their movement through the body takes a few days. Many of them probably get lost and die on their way as witnessed by their presence in the body cavity, in the liver and other organs. They are however found in the place of their further development in a few days.

The changes taking place in the body during that movement are very slight. Having penetrated into the fish the cercaria loses the tail, and while it moves, covering longer distances in paticular, it uses the secretion of the penetrative glands. Major changes occur only in the final place of further development, i.e. in the chorioid coat of the eye or in the brain. The time of passage from the place of penetration to the eye of the brain varies from 20 hours to several days depending on the place of penetration.

The influence of the cercaria on the fry is fatal, particularly when several penetrate at one time. The fish cannot survive one to two days and when heavily invaded by the cercariae they die in several hours. I suppose that the cause of death is the action of the secretion or that of the movement of a young metacercaria through the organism of a small fish involving damage to the internal organs. Consequently when I wanted to preserve the fry I invaded it with a limited number of cercariae, three to eight cercariae per small fish. That number enabled a fish to be invaded several times and keep up to ten parasites in it without any visible damage to the health and life of the fish.

The action of the parasite on small fish must have always been obnoxious despite these precautions above all because of a large difference in the growth rate of the fish and parasite. The parasite grows far faster and so the settling of the cercaria in the retina causes exophthalmus and when it settles in the brain there is pressure on the nervous cells and tissue causing subsequent disturbances (K o z i c k a, 1958). The endurance of the small fish in such cases was surprising in such cases. They continued to live with several large metacercariae encysted and occupying much space in the retina, chorioid coat or brain.

When I found out that the influence of mass invasion was harmful I made experiments with small doses of cercariae only. For this purpose I put one small fish and seven or eight cercariae into a crystallizer (9 × 9 × 4.5 cm) and transferred the fish to an aquarium

in 20 to 30 minutes. I repeated the invasion of the same fish during the following three days. Thus each fish received several cercariae in small doses during four days and could be kept in the aquarium for a month and a half without any distinct harmful action of the cercariae. Two series of experiments were carried out according to this method of invasion.

The first was carried out between July 13th and Sept. 12th, 1953. Twelve young fishes (2.1 to 3 cm long) were used in the experiments. They were invaded between July 13th and 17th and examined between July 28th and Sept. 12th, 1953, the results being presented in Table IIa.

Table IIa

Date of examination	Fish number	Found in	Number of metacercariae	Development condition	Development time
July 28	1	eyes	3 + 2 = 5	Beginning of differentiation of body	
Sept. 5	2	„	6 + 2 = 8	before encystment	
Sept. 5	3	„	3 + 2 = 5	before encystment	
Sept. 5	4	„	4 + 4 = 8	differentiation of body	
Sept. 6	5	„	2 + 1 = 3	before encystment	5—6 weeks
Sept. 8	6	„	5 + 3 = 8	before encystment	
Sept. 9	7	„	7 + 3 = 10	before encystment	
Sept. 9	8	„	5 + 6 = 11	before encystment	
Sept. 9	9	„	7 + 5 = 12	differentiation of body	
Sept. 10	10	„	5 + 4 = 9	differentiation of body	
Sept. 11	11	„	6 + 4 = 10	4 before encystement and 6 encysted	
Sept. 12	12	„	5 + 5 = 10	3 before encystment and 7 encysted	7½ weeks

The second series of experiments I carried out between July 28th and Sept. 14th, 1953. Sixteen fishes (1.7 to 2.3 cm long) were used in the experiments. They were invaded between July 28th and 31th, and examined between Aug. 10th and Sept. 14th, 1953, the results being presented in Table IIb.

The cercariae from the invasion were found in both eyes, as shown in the tables, or sometimes in the brain. They were found between the chorioid coat and the retina or inside either of these screens.

Table IIb

Date of examination	Fish number	Found in	Number of metacercariae	Development condition	Development time
Aug. 10	1	eyes	6 + 5 = 11	1.12 × 0.08 — 0.30 × 0.03	
Aug. 10	2	„	0 + 3 = 3	0.73 × 0.40 — 0.10 × 0.40	
Aug. 12	3	„	7 + 3 = 10	0.10 × 0.09 — 0.34 × 0.07	
Aug. 12	4	eyes & brain	50+30= 80	0.40 × 0.13 — 0.46 × 0.20	
Aug. 17	5	eyes	4 + 3 = 7	growth stage	
Aug. 17	6	„	4 + 5 = 9	differentiation stage	2—3 weeks
Aug. 17	7		the fish died		
Sept. 2	8	brain	7 + 2 = 9	before encystment 2 encysted	4—5 weeks
Sept. 4	9	eyes	2 + 2 = 4	encysted	
Sept. 10	10	„	3 + 4 = 7	6 before encystment and 1 encysted	
Sept. 10	11	„	1 + 0 = 1	before encystment	
Sept. 12	12	„	4 + 4 = 8	before encystment	
Sept. 14	13	„	2 + 4 = 6	before encystment	6—7 weeks
Sept. 14	14	„	1 + 3 = 4	before encystment	
Sept. 14	15	„	2 + 2 = 4	before encystment	
Sept. 14	16	„	2 + 1 = 3	before encystment	

The experiments show that:

(a) 27 out of 28 fishes yielded a positive result of the experiment. The result was unknown in one case only, because the fish died and could not be experimented on.

(b) The cercaria coming from *Planorbis planorbis* is the first larval form of *Posthodiplostomum brevicaudatum.*

(c) A single invasion does not immunize the fish, they could ğet invaded two or more times. This is proved by a higher or similar number of cercariae than that used in single experiments. Allowances must be also made for a certain percentage of cercariae which must have undoubtedly died during the experiments and the movement through the organism of the fish.

(d) Invasion by a high number of cercariae causes a serious shock in a fish resulting in death. This is brought about probably by the action of the secretion of the penetrative glands or mechanical damage to the tissue caused by the larvae moving through the organism.

(e) The development of the cercaria of *P. brevicaudatum* into a metacercaria in the fish and probably of other related metacercariae takes seven to eight weeks in summer.

The development stages of the metacercaria

When penetrating into the fish the cercaria loses its tail. The wandering stage begins in the fish during which I noticed no changes in the metacercaria. This stage lasts till the metacercaria reaches the retina or the chorioid coat of the eye. When it reaches the site where it occurs regularly it starts a stationary life, for it can pass hence only to the brain along the optic nerve. This happens seldom and, I believe, depends on the occurrence of more specimens in the eye.

Only when the screens of the eye have been reached the first changes start which open the metacercaria period. Among these changes it is possible to distinguish the successive stages of growth, of the differentiation of various sections of the body, the stage before the encystment and that of encystment. The changes of the metacercaria period concern not only the morphological and anatomical features but also the physiological properties.

The changes are opened by a period of growth. The body becomes intransparent and much granulation appear in it which make the tissue dark. The granulation appears along the main excretory canals and thus very roughly outlines their course. With the advancing process of the appearing granulation its arrangement in the body becomes less regular and the body less transparent. The frontal organ, part of the alimentary tract, eyes and glands all become invisible and only in some forms the remains of the ducts of the penetrative glands may be seen in the terminal section of the head. The cercaria still preserves a great vitality and mobility, elongates, twists, narrows and widens till it becomes oval in shape.

I first supposed that the appearance of the granulation in the body, the distinctly diminished transparence of the tissue and the disappearance of some organs all reflected a histolytic process. But the total preparations showed that the number of cells in the body considerably increased at that time. This might be the reason for the poor transparence of the tissue. As the number of cells increases the dimensions of the body grow and amount to $0.12 \times 0.08 — 0.50 \times 0.20$ mm, and so I think it legitimate to interpret this period as one of growth. It is marked by the accumulation of elements for the development of a new organism. It takes two to three weeks from the invasion of the fish (Tables IIa & IIb).

The period of the differentiation of the body is characterized by the disappearance of the granulation which is probably removed

through the excretory canals outside. This brings about some clearing up of the tissue. At the same time new organs such as the oral sucker, the ventral sucker and the adhesive organ begin to appear. The section of the body does not undergo any major changes, it is still consistently rod-like in shape, widens and narrows here and there (Fig. 3). The oral sucker measures 0.03 — 0.05, the ventral sucker 0.03 mm. The adhesive organ is round or elliptical in outline (0.08 × × 0.08 mm). Muscular cells form in the oral sucker and so make it differ from the frontal organ. In that period, the adhesive organ differ morphologically little from the ventral sucker and may be mistaken for a sucker even more easily as the latter is small and very difficult to see in a live metacercaria.

After four weeks' development in the fish the metacercaria reaches a length of 0.60 × 0.15 — 1.57 × 0.40 mm. It does not differ basically from the previous period except for distinct anlages. The body is still elongated and rod-like or gets shorter through contractions and becomes elliptical in shape, yet processes slowly begin in it leading up to a distinct change of the shape of the body. The beginning of these processes is at first a change in the character of the fore and hind sections of the body. The number of excretory canals begins to grow in the fore section including the adhesive organ. Three to five canals appear in the dark mass of the granulation; they have granular content and are connected with each other. Thus, an increasing number of canals may be seen. Smaller grains coalesce into bigger ones and thus excretory bodies are formed adipose in character; their adipose composition is suggested above all by their dissolubility under the action of alcohol.

As the larva grows there are more meshes in the excretory net and consequently the fore section of the body differentiates (Fig. 4).

Changes also take place in the hind section. Large vesicles appear in it and small grains accumulate among them. The vesicles are bright, swollen and look like cells with degenerating plasm (Fig. 4). The whole assumes the character of a delicate tissue little resistant to the action of water. There starts the stage of the differentiation of two sections of the body, fore and hind, differing from each other in character. There is still no morphological division between them because the movement of the body effaces the differences between them. Rather unexpectedly and suddenly the body becomes distinctly narrow at a point and a furrow across it divides the fore section

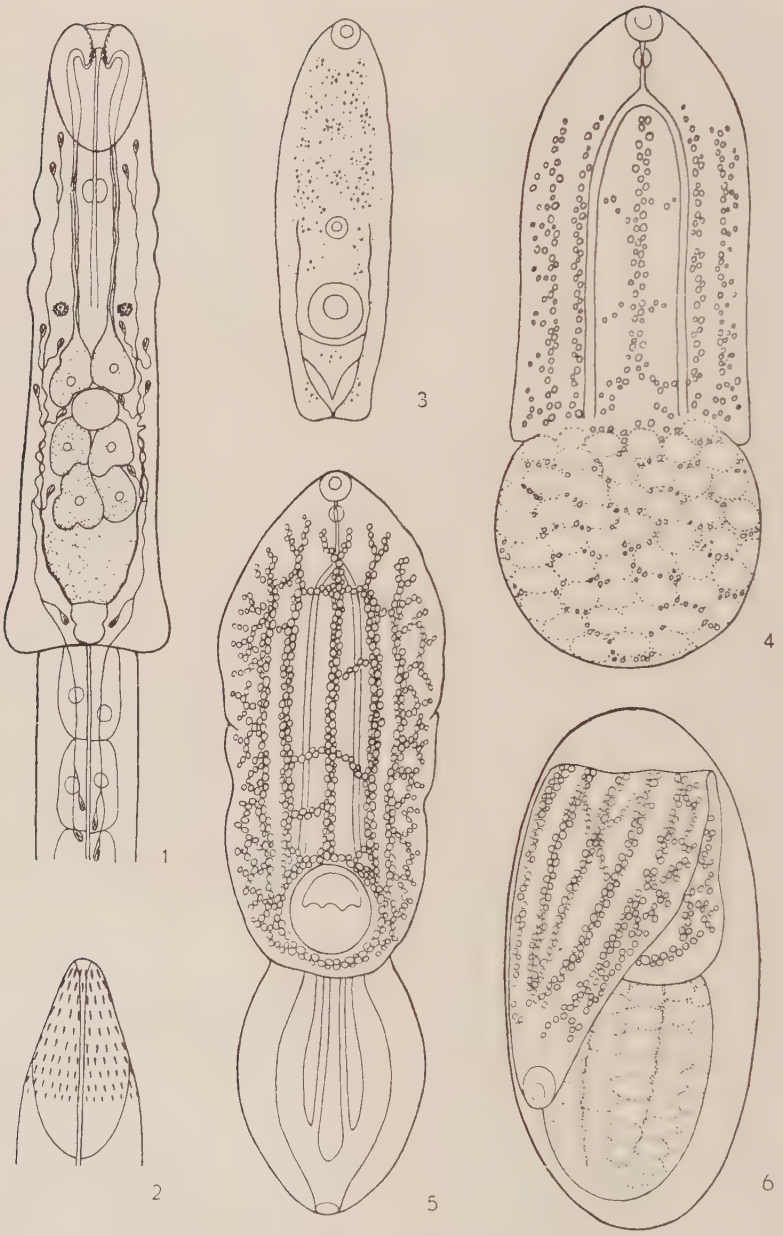

Fig. 1-6. Fig. 1. Cercaria *Posthodiplostomum brevicaudatum*. Fig. 2. Armature of head of cercaria. Fig. 3. The beginning of the period of the differentiation of the body (0.5 × 0.2 mm). Fig. 4. The period of the differentiation of the body (ca 1 mm long). Fig. 5. Mature metacercaria *P. brevicaudatum*. Fig. 6. Mature metacercaria encysted.

from the hind one (Fig. 4). The body reaches about 1 mm in length then and divides into a darker and longer fore section (ca 0.62 × × 0.40 mm) and a brighter and somewhat shorter hind section (ca 0.40 × 0.40 mm). Meanwhile an alimentary tract forms and suckers as well as an adhesive organ develop in the fore section, and the anlages of the reproductive organs begin to appear in the hind section. Later on the division into the sections becomes more distinct, particularly when the terminal part of the fore section bends ventrally and forms a fairly thick fold (Fig. 5, 6, 7 & 8).

The body remains in the eye screens free and cystless. During the subsequent period there is a steady growth of the body and a cyst forms. The material of which it is formed is probably supplied by the connective tissue of the host which surrounds it reacting to the movements of the parasite. This is suggested by there being no special cystigenous glands in the metacercaria. The encystment gives the metacercaria different physiological properties and makes it proof against the influence of the external environment. Before encystment metacercariae quickly decompose when removed from the eye. The body tissue cannot stand contact with water, encysted metacercariae however keep in water for a very long time without losing anything of their vitality.

The body of the metacercaria may be easily removed from the cyst. The cyst varies from 0.93 × 0.46 to 1.24 × 0.38 mm, it is elastic, their walls extend with the movement of the fluke or change otherwise. It does not change any of its dimensions during the life of the metacercaria unless their walls become thicker. But the larva inside it grows and after a long stay in the host becomes considerably longer than the cyst (Fig. 6). Its size varies between 0.93 and 1.10 mm. The volume of the cyst is large and so the larva has much room to move in it and turns about fairly briskly. Later on it fills up the inside and restrained by little space bends either dorsally or ventrally (Fig. 6). A fully developed larva differs little from the stage described. The anlages of the reproductive organs grow in it and the copulative organ develop.

The body of a well-developed metacercaria is always divided into two sections. The fore section is like a leaf in shape, the hind one is shorter and only somewhat narrower and oval (Fig. 5). The sides of the body partly bend centrally in the fore section and consequently this section is concave like a basin (Fig. 7 & 8). The body is large:

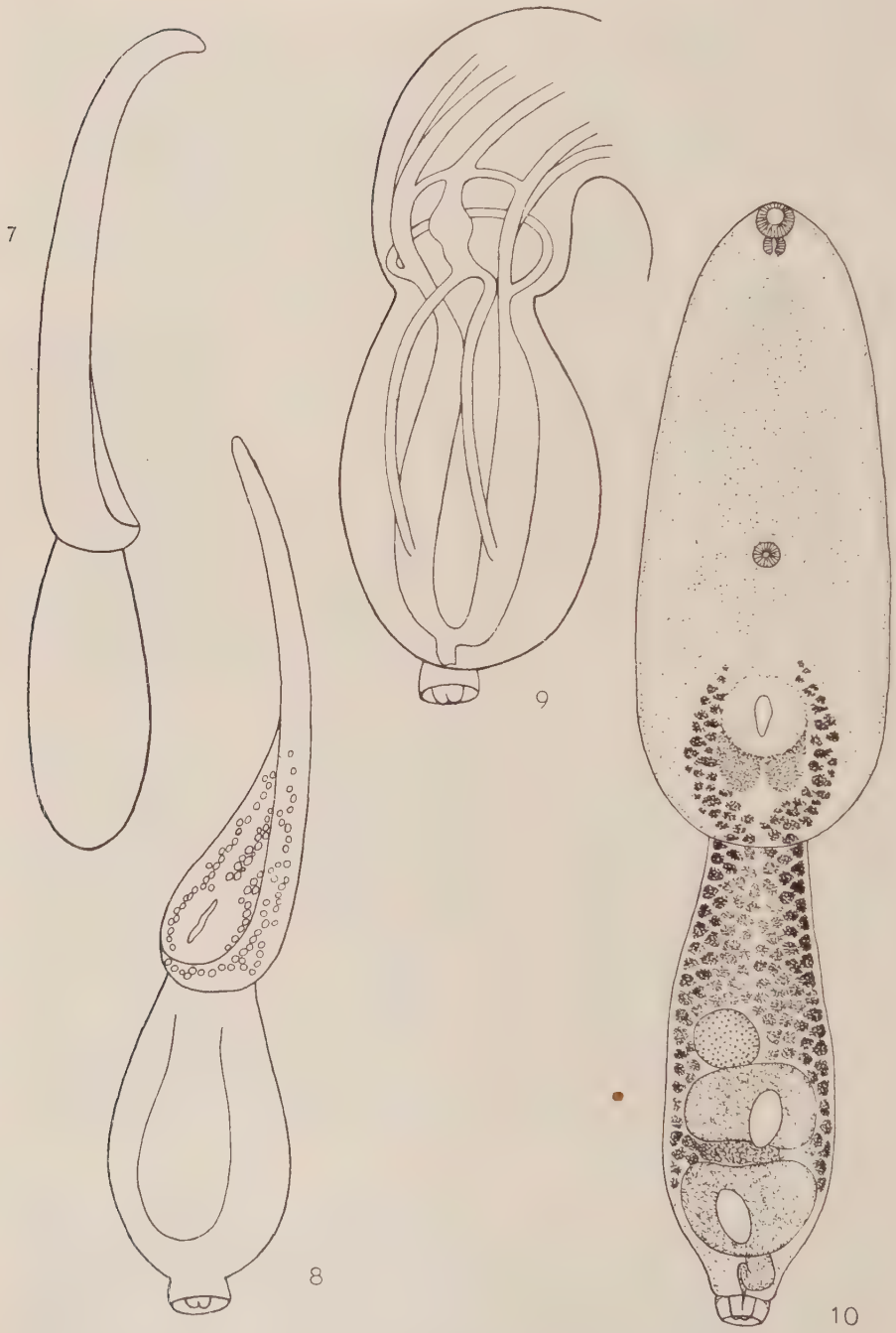

Fig. 7-10. Fig. 7 and 8. Metacercariae removed from cyst. Side view. Fig. 9. Excretory system; terminal section. Fig. 10. Final form of *P. brevicaudatum* from *Botaurus stellaris*.

0.50 — 0.80 × 0.35 mm. The hind sections contains many vesicular bodies which dissolve under the action of alcohol. The excretory system varies from the fore section to the hind one. The accumulation of a large number of excretory bodies in the fore section lends a darker hue to this part of the body. The excretory vesicle at the end of the hind section has the shape of a small fork. From both its points two large canals go towards the front of the body, penetrate it and pass into the net of the canals running in the fore section. Besides, from the hind sections of the large canals two narrower canals go which pass on to the dorsal side when reaching the fore section and join each other into a central vessel running across the middle of the fore section (Fig. 5). The canals of the fore section consist of three to five vessels filling up the centripetal part of the fore section and of the lateral network consisting of minute canals and with considerably smaller meshes, the arrangement of the network thus becoming irregular. The adhesive organ becomes more elliptical in shape. Its borders are folded and there already appear glandular cells in its walls; these cells stain more strongly, above all in the centripetal parts and this may be seen from two larger accumulations of the pigment in preparations.

The mature form

I found a mature form in one specimen of *Botaurus stellaris* (37 flukes) and in one *Ixobrychus minutus* (8 specimens).

The number of metacercariae found in fish suggests that *P. brevicaudatum* in the biocoenosis of Lake Drużno is more common than in that of Lake Mamry Północne. A criterion of frequency based on the number of metacercariae found in fish is in this case more realistic than one based on the number of flukes found in the final hosts as the latter live a stealthy life and are difficult of access. Accordingly, when examining the distribution of metacercariae in the biocoenosis of fish we base ourselves on more objective comparisons.

The differences between a metacercaria and a mature form consist above all in a different size and shape of the hind section. No differences between a metacercaria and a mature fluke may be seen in specimens which have recently got from a fish into a brid. The relations and proportions between the fore and hind sections are the same. Specimens which had to remain in the final host for a long time

have a slight body increase, particularly in the hind section, which changes the mutual proportions of the body sections.

The body increase in the final host takes place chiefly in the hind section and this changes the relation of both sections to each other. While the fore section is always somewhat longer than the hind one in the metacercaria, both sections are almost equal in the mature form, the hind section narrowing above all where it borders on the fore section (Fig. 4,5 & 10).

Below is a comparison of the body dimensions of the metacercaria and the mature form. On the whole the specimens coming from *Ixobrychus minutus* are somewhat smaller than those from *Botaurus stellaris*.

Metacercaria	Mature form
$0.63 \times 0.48 + 0.23 \times 0.31 = 0.86$	$0.45 \times 0.22 + 0.30 \times 0.16 = 0.75$
$0.68 \times 0.53 + 0.42 \times 0.43 = 1.10$	$0.53 \times 0.32 + 0.53 \times 0.16 = 1.06$
$0.70 \times 0.42 + 0.48 \times 0.41 = 1.18$	$0.64 \times 0.31 + 0.68 \times 0.26 = 1.32$
$0.91 \times 0.45 + 0.45 \times 0.42 = 1.36$	$0.76 \times 0.36 + 0.71 \times 0.22 = 1.47$
$1.07 \times 0.54 + 0.48 \times 0.45 = 1.55$	$0.91 \times 0.30 + 0.68 \times 0.13 = 1.59$
	$1.07 \times 0.14 + 0.80 \times 0.22 = 1.87$

The fore section has the shape of a leaf and is widest at the adhesive organ from which it distinctly narrows running forward. The hind section is widest at the hind testicle and narrows more abruptly towards the front than towards the end of the body (Fig. 10).

Except for the part of vitelline glands the reproductive organs lie in the hind section. The vitelline glands cover the portion from the level of the adhesive organ (or stretch even farther towards the front) to the fore level of the second testicle. They apear more densely and in larger numbers in the hind section. They also get between the first and second testicles, where they form a large accumulation even more distinct as not covered by any other organ.

The ovary is smaller than either testicle. It lies just in front of the first testicle, touches and may even partly cover it. The hind testicle is somewhat larger than the fore one and lies at a certain distance from it. Part of the vitelline glands force their way in the free space between. The testicles are oval. Large eggs (0.10 × 0.07 mm), one or two in number, occur in the uterus.

At the end of the body there is a copulative apparatus of a characteristic structure, after which D u b o i s gave it the taxonomic name of *Posthodiplostomum impraeputiatum*. The structure of the

copulative apparatus is fully completed and this helped identify the metacercaria with the mature form. I often observed the metacercariae with their copulative apparatus thrust outside. It protrudes then like a bell with the conus genitalis sticking out in ist middle. The conus genitalis may be partly or completely hidden or extruded. When it is completely hidden, the bursa copulatrix sticks out like a hollow bell. The bell may be withdrawn into the atrium genitale and the termination of the body is oval then. The bursa copulatrix is generally thrust outside.

The adesive organ, lying in the hind part of the fore section, is oval, nearly spherical (0.15—0.21 mm). The glands making up its content extend it however towards the main axis of the body.

The suckers are small, indistinct, difficult to trace. The oral sucker is somewhat larger than the ventral sucker, their size being 0.03 — 0.04 and 0.03 mm respectively. The latter lies in the middle of the fore section.

Comments for discussion

It has been long known that the eyes of fish are an important place for various species of the larvae of the *Strigeidae* to develop at. When she investigated the fish parasitofauna of the Mazury Lake District, Kozicka found *Diplostomulum volvens* (Nordmann) Faust, *Diplostomulum conifera* Kozicka, *Diplostomulum clavatum* (Nordmann) Faust, *Posthodiplostomulum brevicaudatum* (v. Nordmann) Kozicka, *Neascus scardinii* Schulmann and others in the eyes. Above all, *Posthodiplostomulum* and *Neascus,* morphologically and anatomically similar to each other, are noteworthy. The larva *Neascus* was created by Hughes when he described *Neascus ambloplitis* Hughes, 1927. Dubois (1927) separated the larva *Posthodiplostomulum* to denote the metacercaria stages of *Posthodiplostomum* and reserved the name *Neascus* for the metacercariae of the genera *Crassiphialini.* I do not challenge Dubois's point of view that *Posthodiplostomulum* is the metacercaria of *Posthodiplostomum,* and *Neascus* the metacercaria of the species of the subfamily *Crassiphialini,* but I cannot at the moment see any possibility to establish distinct anatomical differences between these two larvae. The difference in the structure of the bursa copulatrix, suggested by Dubois, distinct in mature forms, cannot be observed in metacercarial ones. The difference is supposed to consist in the

occurrence in the end of the hind section of the body of *Posthodiplostomulum* of an extrusive bursa copulatrix, which never occurs in the representatives of *Crassiphialini*. As regards the metacercaria *Uvulifer ambloplites* however the drawing by H u g h e s (1927, Plate V, Fig. 1, Plate VI, Fig. 3) and his description "a small bursa copulatrix sometimes extruded from the dorsal side of the posterior end of the hind body" offers a picture difficult to distinguish from the bursa copulatrix in *Posthodiplostomulum brevicaudatum*, and so there is no reliable criterion to make it possible to distinguish these types of larva from each other.

The distinction between the larvae *Neascus* and *Posthodiplostomulum* cannot be so important from the point of view of tracing separate development lines as is the development line of *Tetracotyle, Diplostomum* or *Prohemistomulum* which also determine the natural affinity of the mature species belonging to them. Only the *Neascus* s. l. larval type can be put together with these types of larva as one representing a definite development trend. It would also include *Posthodiplostomulum, Ornithodiplostomulum* and possibly other related larvae identified as metacercariae of the species *Posthodiplostomum, Ornithodiplostomum, Uvulifer, Crassiphiala* and possibly others. Given a common name of *Neascus* these genera would seem to be related to each other and follow one and the same development line.

We know today the development cycle of the following related species — see Table II.

In addition to the cercariae already connected into life cycles, those should be mentioned whose further development has not been closely determined and which are related to the above fluke group through their features. These are *C. flexicorpa* C o l l i n s, 1935, *C. hamata* M i l l e r, 1923, *C. rhabdocaeca* F a u s t, 1915. The structure of all these cercariae is not determined or known accurately enough to distinguish among them cercariae belonging to *Posthodiplostomulum* or other larvae. The cercariae however differ from other *Furcocercariae* in that they have no well-developed ventral sucker but only a group of cells as an anlage of this sucker, three pairs of penetrative glands and underdeveloped eye pigment. The cercariae thus distinguished may be regarded as the first larval forms of *Neascus* s. l.

Table III

Mature form	Metacercaria	Cercaria
1. *Posthodiplostomum minimum* (MacCallum, 1921) Dubois, 1936	*Neascus van-cleavei* (Agersburg) Hughes, 1928 (*N. oneidensis*)	*Cercaria multicellulata* Müller, 1923
2. *Posthodiplostomum brevicaudatum (impreputiatum)* Dubois, 1936	*Posthodiplostomulum brevicaudatum* (v. Nordm.) Kozicka, 1957	*P. brevicaudatum* mihi
3. *Posthodiplostomum cuticula*	*Posthodiplostomulum cuticula* (Nordmann, 1932) Dubois, 1937	unknown
4. *Crassiphiala bulboglossa* Haitsma, 1925	*Neascus bulboglossa* (v. Haitsma, 1928)	unknown
5. *Uvulifer ambloplites* (Hughes, 1927) Hunter III 1933	*Neascus ambloplites* Hughes, 1927	*Cercaria bessiae* Cort et Brooks
6. *Ornithodiplostomum ptychoheilus* (Faust, 1917) Dubois, 1936	*Ornithodiplostomulum ptychoheilus* (Hughes et Piszczak, 1928) Dubois, 1938	unknown

It follows from the above that four main larval types occur among the *Strigeidae — Tetracotyle, Diplostomulum, Neascus* and *Prohemistomulum*. Their respective cercariae are connected with them as their forms of departure on the one hand and mature forms on the other. These are types of larvae representing definite directions in the philogenetic development. Each of them has in turn representatives corresponding to various species. Thus, *Tetracotyle* corresponding to *Cotylurus, Apatemon, Ophiosoma* (called *Codonocephalus*) may be distinguished among *Tetracotyle*, there are larvae belonging to the species *Diplostomum* and *Tylodelphys* among *Diplostomulum*, the larvae of *Cyathocotyle, Cyathocotyloides, Linstowiella, Prohemistomum* and others among *Prohemistomulum* and the larvae *Posthodiplostomulum, Ornithodiplostomulum, Uvulifer, Crassiphiala* and others among *Neascus*. It would be desirable to work out proper definitions for these larval types and above all give a closer characterization of the corresponding larvae, i. e, cercariae and metacercariae.

As regards *Neascus* s. l. I agree with the definition of Hughes (1927) except that I think that the interpretation of the so called reserve bladder in the excretory apparatus should be changed. The excretory system was described in fair detail by Hughes in *Neascus ambloplites* and by W. S. Hunter in *N. wardi*. As interpreted by these authors the excretory system consists of the primary part and the reserve bladder only loosely connected with the primary system. The primary system corresponds to the canals and flames observed in the cercaria. It is not seen in the metacercaria as it is covered by a system of canals making up the reserve bladder. On the evidence of *P. brevicaudatum* I do not think that the large canals of the fore and hind section of the body correspond to the reserve bladder independent of the original system with which it is said to have a secondary connection. The canals filled up with bodies are extensions of the primary system of canals and canaliculi originating from the vibratory flames. They lie deep inside the parenchymatous tissue and that is why they cannot be seen. The canals of the so called reserve bladder run from the excretory vesicle proper and are a further complicated development of the primary and secondary canals in the cercaria. This is borne out by the arrangement observed in the metacercaria which corresponds to the secondary canals in the cercaria reaching the excretory vesicle. Neither did I observe any circular canaliculi in *P. brevicaudatum* connecting the network of canals of the reserve bladder which run under the cuticle on both sides of the hind section. As interpreted by the above authors, the system of canaliculi in the hind section is very complicated and does not coincide with what is to be found in *Posthodiplostomum brevicaudatum*.

In this connection a definition of the larva *Neascus* s. l. may be formulated as follows: it is the metacercaria of the *Strigeidae* with two distinct sections of the body, fore and hind, without pseudosuckers or lateral sacs in the fore section, with an oval adhesive organ, with well-developed canals of the excretory system filled with numerous granules; it lives encysted in various organs (eyes, liver, muscles, body cavity, etc) in fish.

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STRESZCZENIE

Autor znalazł u *Botaurus stellaris* (bąk) i *Ixobrychus minutus* (bączek), pochodzących z biocenozy ptaków jeziora Drużno i Mamry Północne, przywrę, którą oznaczył jako *Posthodiplostomum impraeputiatum*. W biocenozie ryb jezior Drużno, Gołdapiwo i Mamry Północne znajdowała Kozicka *Posthodiplostomulum brevicaudatum* (metacerkarię). Autor zidentyfikował metacerkarię z ryb z postacią dojrzałą z *Botaurus stellaris* i *Ixobrychus minutus*, wobec czego nazwa przywry powinna brzmieć *Posthodiplostomum brevicaudatum* (v. Nordmann, 1832) Kozicka, 1958.

Autor przeprowadził cykl rozwojowy i stwierdził następującą kolejność żywicieli: cerkaria żyje u *Planorbis planorbis* i należy do furkocerkarii. Metacerkaria rozwija się w narybku różnych gatunków ryb *(Leuciscus rutilus, Perca fluviatilis)*, a zwłaszcza u *Scardinius erythrophthalmus*, w siatkówce i naczyniówce oka oraz w mózgu. Ostatecznym żywicielem jest bąk i bączek.

Autor podał opis cerkarii, metacerkarii, postaci dojrzałej i w części dyskusyjnej uwagi na temat typów larw u *Strigeidae*.